Scandinavian Journal of Infectious Diseases, 2010; 42: 650–657

informa
healthcare

ORIGINAL ARTICLE

Kinetics of the QuantiFERON®-TB Gold In-Tube test during treatment of patients with sputum smear-positive tuberculosis in relation to initial TST result and severity of disease

JONNA IDH[1], EBBA ABATE[1,2,3], ANNA WESTMAN[4], DANIEL ELIAS[3], HELENA JANOLS[5], ASCHALEW GELAW[2], ASSEFA GETACHEW[2], SHITAYE ALEMU[2], ABRAHAM ASEFFA[3], SVEN BRITTON[4], OLLE STENDAHL[1] & THOMAS SCHÖN[1,6]

[1]*Department of Medical Microbiology, Faculty of Health Sciences, Linköping University, Linköping, Sweden,* [2]*Gondar College of Medical and Health Sciences, Gondar University, Gondar, Ethiopia,* [3]*Armauer Hansen Research Institute, Addis Ababa, Ethiopia,* [4]*Department of Infectious Diseases, Karolinska Hospital, Stockholm, Sweden,* [5]*Department of Infectious Diseases, Malmö University Hospital, Malmö, Sweden, and* [6]*Department of Clinical Microbiology, Kalmar County Hospital, Kalmar, Sweden*

Abstract

The QuantiFERON®-TB Gold In-Tube test (QFN) measures interferon-gamma production in response to Mycobacterium tuberculosis antigens. Our aim was to assess the kinetics of the QFN and initial tuberculin skin test (TST) result in relation to severity of disease in a tuberculosis (TB) endemic area. Smear-positive TB patients (n = 71) were recruited at Gondar University Hospital, Ethiopia. The TST, QFN, CD4+ cell count and clinical symptoms (TB score) were assessed and followed up during treatment. From baseline to 7 months after treatment, there was a significant decrease in QFN reactivity (93.8% to 62.5% in HIV-negative/TB; 70.3% to 33.3% in HIV-positive/TB patients) down to a level comparable to a control group of blood donors (51.2%). The agreement between TST and QFN was poor in TB patients compared to healthy controls. A negative TST correlated to more advanced TB in contrast to a negative QFN test. We conclude that the QFN reactivity is significantly reduced at the end of treatment against active TB to the background level of healthy blood donors, and that the agreement between TST and QFN is poor including correlation to the severity of disease.

Introduction

The tuberculin skin test (TST) has been used as a diagnostic tool for tuberculosis (TB) since the 19th century and it has a high specificity in non-BCG (bacille Calmette–Guérin) vaccinated populations, but markedly reduced specificity in BCG vaccinated individuals. Furthermore, the TST is associated with low sensitivity in people with impaired cellular immunity and has logistical drawbacks, i.e. patients need to return to the healthcare facility for the result to be recorded [1].

In recent years, interferon-gamma release assays (IGRAs) measuring in vitro T-cell interferon gamma (IFN-γ) production in response to antigens that are highly specific for Mycobacterium tuberculosis have been developed. The IGRAs are not affected by BCG vaccination and the specificity for M. tuberculosis infection is high for the 2 commercially available IGRAs, QuantiFERON®-TB Gold In-Tube (QFN) and T-SPOT.TB. The sensitivity of IGRAs and the TST is not consistent across tests and populations, but T-SPOT.TB appears to be slightly more sensitive than QFN and TST, at least when comparing to active TB. The lack of a gold standard for diagnosing latent TB complicates the interpretation of such studies, since the tests are rarely directly compared [2].

In high endemic countries for TB, IGRA positivity in the healthy population is reported to be in the range of 30% to 60% both in HIV-infected and HIV-uninfected individuals, reflecting an expected

Correspondence: J. Idh, Department of Medical Microbiology, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden. Tel: +46 703 969897. E-mail: jonna.idh@liu.se

(Received 29 October 2009; accepted 29 March 2010)

ISSN 0036-5548 print/ISSN 1651-1980 online © 2010 Informa Healthcare
DOI: 10.3109/00365548.2010.482942

high prevalence of latent TB in communities with a high level of ongoing transmission [3]. In low endemic areas, the IGRAs are useful in distinguishing latent TB infection from a positive TST due to BCG vaccination [4].

The limited studies conducted on HIV co-infected smear-positive TB patients do not always agree on the impact of immune suppression and low CD4+ cell counts on the sensitivity of the QFN test and possible increase in the number of indeterminate results as a result of a generally low IFN-γ response [5–7]. Furthermore, little is known about the kinetics of QFN results during anti-TB treatment in relation to severity of the disease in high endemic areas. Available studies have shown a moderate reduction in the rate of positive QFN results following treatment and a correlation to increased sputum conversion [8–11], but few follow-up studies have investigated the correlation to clinical outcome and severity of the disease. Moreover, little is known about the reasons and clinical correlates of the reported discordance between IGRAs and the TST in active TB [12]. A clinical scoring system for evaluating severity of disease – the TB score – has recently been developed and consists of a combination of clinical symptoms and signs of TB. This score has been validated and can be used to monitor TB patients during treatment and to assess clinical outcome and severity of disease in a standardized way, as a high TB score is associated with an increased mortality [13].

In this study, the aim was to investigate the kinetics of the QFN test during the treatment of patients with smear-positive TB in relation to the initial TST result and severity of disease.

Materials and methods

Study subjects and design

Consecutive patients with newly diagnosed smear-positive TB ($n = 71$) were recruited at the directly observed treatment short-course (DOTS) clinic of Gondar University Hospital, Ethiopia. The inclusion criteria were age 15–60 y and acid-fast bacilli (AFB) sputum smear-positive TB. The exclusion criteria were hospitalization, pregnancy, or concomitant disease other than HIV. Smear-positive pulmonary TB was defined as 2 out of 3 morning sputum samples positive for AFB or 1 out of 3 positive with a chest X-ray and clinical symptoms suggestive of active pulmonary TB. Sputum AFB status was examined by microscopy and recorded at baseline, 8 weeks and 7 months after initiating treatment. Sputum conversion was defined as 3 consecutive sputum smears turning negative for AFB. Treatment outcome was recorded for all patients at the end of treatment according to the World Health Organization guidelines. According to these guidelines, the definition of 'cured' is a patient who was smear-positive at the start of treatment, completed treatment and who was smear-negative at the end of treatment and on 1 previous occasion. A community control group of blood donors was recruited at the blood bank, Gondar University Hospital. Eligible study subjects were interviewed and were not included in the study if they had previously been treated for TB or had a household contact under treatment for TB. A clinical examination as well as an interview was performed with each community control to exclude any concomitant diseases including HIV, syphilis and other chronic illnesses as a part of the routine at the blood bank. In all study subjects the presence of a BCG scar and body mass index (BMI) were registered. The TST response was measured after blood was drawn for the analysis of QFN, CD4+ cell count and HIV. CD4+ cell counts were obtained using a FACSCount instrument (BD, San Jose, California, USA). The initial CD4+ cell count was measured within 2 weeks of treatment initiation. All patients and study subjects were offered pre- and post-test counselling at the voluntary counselling and testing (VCT) clinic prior to HIV testing according to the hospital routine. HIV-positive patients were referred for treatment to the anti-retroviral therapy (ART) clinic, available at the hospital. This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Research and Publication Office of the University of Gondar, Gondar College of Medical and Health Sciences, Ethiopia and the Regional Ethics Review Board, Linköping, Sweden. All patients and study subjects were included only after obtaining written informed consent.

Clinical data

According to the TB score, signs and symptoms were recorded as previously described [12]. The presence or absence of clinical symptoms including cough, haemoptysis, dyspnoea, chest pain and night sweating were recorded, as well as clinical signs and other parameters including anaemia (paleness of the conjunctivae at eye examination), tachycardia, lung auscultation findings, fever, BMI (<16 or <18 kg/m^2) and mid-upper arm circumference (MUAC <200 or <220 mm), resulting in a TB score from 0 to 13. By dividing the patients based on the initial score, patients were distributed into 3 severity classes (SC): SC-I, a TB score of 0–5; SC-II, a TB score of 6–7; and SC-III when the TB score was ≥ 8 points [12].

HIV serology

The HIV status of the TB patients was analysed with Enzygnost Anti-HIV 1/2 Plus (Dade Behring,

Germany) and confirmed with Vironistika HIV Uni-Form II Ag/Ab (Biomérieux, France) using an enzyme-linked immunosorbent assay (ELISA) multi-well reader (Anthos Labtech instrument 2001, Austria). The blood donors were analysed according to the hospital routines with the Vironistika HIV Uni-Form II Ag/Ab micro-ELISA system.

QuantiFERON®-TB Gold In-Tube assay (QFN)

Blood samples were collected in tubes provided by the manufacturer of the QFN test (Cellestis). QFN is an in vitro diagnostic test that uses a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7 (p4) proteins to stimulate cells in heparinized whole blood. At inclusion, after 2–3 months and after 7 months of anti-TB treatment, a QFN test was done according to the manufacturer's instructions (Cellestis). All QFN samples were collected in the morning at 09.00 a.m.–12.00 p.m. One millilitre of blood was collected into each of the 3 QFN blood collection tubes. At the time of sample collection and prior to incubation, the samples were mixed thoroughly by shaking the tube 10 times (5 s) to ensure that the entire inner surface of the antigen coated tube was covered with the blood. The samples were transported and incubated at 37°C at 12.00 p.m. for 18–22 h after which the serum was frozen at −20°C for later analysis.

Tuberculin skin test (TST)

Two tuberculin units of purified protein derivative (PPD; Statens Serum Institute (SSI), Copenhagen, Denmark) were injected intradermally on the ventral aspect of the lower arm. The diameter of the skin induration was measured 48–72 h later according to the manufacturer's instructions (SSI, Denmark). A diameter of ≥10 mm was considered positive.

Statistics

Data are presented as mean ± standard deviation (SD). To compare groups, the Student's *t*-test was used for parametric data and the Chi-square test for discrete variables. A p-value of <0.05 was considered statistically significant. Kappa was calculated according to Cohen. Significant variables were entered into a multiple logistic regression model using the STATISTICA software package (StatSoft, Tulsa, USA).

Results

Clinical data

Seventy-one sputum smear-positive TB patients were included in the study (Table I). Fifty-four percent ($n = 38$, mean age 32 ± 10 y) were HIV-positive (HIV-positive/TB) and 46% ($n = 33$, mean age 25 ± 6 y) were HIV-negative (HIV-negative/TB). A control group of blood donors ($n = 41$, mean age 27 ± 8 y, all male) without a history of TB or any household contacts on TB treatment was recruited. Among HIV-negative/TB patients, 27/33 (82%) completed 8 months of treatment, none died, 1 defaulted and 5 patients were transferred out. Among HIV-positive/TB patients, 27/38 (71%) completed 8 months of treatment, 2 patients defaulted, 8 died and 1 was transferred out. The presence of a BCG scar was low in general (13% among HIV-negative/TB and 24% among HIV-positive/TB patients). No difference in the severity of clinical symptoms and signs measured as the TB score was found between HIV-positive/TB and HIV-negative/TB patients at inclusion. HIV-positive/TB patients had a significantly lower CD4+ cell count at inclusion compared to HIV-negative/TB patients (219 ± 154 vs 561 ± 283 cells/μl, $p < 0.01$). Healthy blood donors had a significantly higher CD4+ cell count compared to HIV-negative/TB patients ($p < 0.001$, Table I).

Table I. Baseline characteristics of TB patients and community controls.

Characteristic	HIV-neg/TB $n = 33$	HIV-pos/TB $n = 38$	p-Value	TB total $n = 71$	Controls $n = 41$
Age, y	25 ± 6	32 ± 10	<0.01	28.8 ± 9.1	27 ± 8
Sex	15F, 18M	14F, 24M	NS	29F, 42M	0F, 41M
Body mass index, kg/m^2	16.3 ± 1.6	16.7 ± 2.0	NS	16.5 ± 1.9	20 ± 2
TB score	7.6 ± 2.3	7.4 ± 2.1	NS	7.5 ± 2.2	0.63 ± 1.1
BCG scar	13.3% (4/30)	24.2% (8/33)	NS	19.0% (12/63)	10.3% (4/39)
CD4+ cell count (cells/μl)	561 ± 283	219 ± 154	<0.01	375 ± 280	786 ± 218
TST (mm)	18 ± 8	15 ± 10	NS	16 ± 9	7 ± 8
TST ≥10 mm	87.9% (29/33)	73.0% (27/37)	NS	80.0% (56/70)	47.2% (17/36)
QuantiFERON: positive	93.8% (30/32)	70.3% (26/37)	<0.05	81.2% (56/69)	51.2% (21/41)
QuantiFERON: negative	6.2% (2/32)	27.0% (10/37)	<0.05	17.4% (12/69)	43.9% (18/41)
QuantiFERON: indeterminate	0.0% (0/32)	2.7% (1/37)	NS	1.4% (1/69)	4.9% (2/41)

Data are presented as mean ± standard deviation.
HIV, human immunodeficiency virus; TB, tuberculosis; M, male; F, female; BCG, bacille Calmette–Guérin; TST, tuberculin skin test; NS, not significant.

The impact of HIV and CD4+ cell count on QFN and TST

Defining a positive TST as ≥10 mm, 87.9% of HIV-negative/TB patients and 73.0% of HIV-positive/TB patients had a positive TST when diagnosed with TB, compared to the QFN test where 93.8% of HIV-negative/TB patients and 70.3% of HIV-positive/TB patients were positive, giving an overall sensitivity for smear-positive pulmonary TB (HIV-positive and HIV-negative/TB) of 80.0% for the TST and 81.2% for QFN including indeterminate results in the denominator (Table I).

A CD4+ cell count < 200 cells/μl significantly affected the rate of patients positive in the TST (61.1% (11/18) with a CD4+ cell count < 200 cells/μl vs 85.7% (42/49) with a CD4+ cell count > 200 cells/μl, $p = 0.03$), whereas QFN-positive patients exhibited a non-significant difference (72.2% (13/18) with a CD4+ cell count < 200 cells/μl vs 91.5% (43/47) with a CD4+ cell count > 200 cells/μl, $p = 0.10$). The lowest CD4+ count recorded was 30 cells/μl and this subject had a positive QFN test. A detailed presentation of QFN and TST results in relation to CD4+ counts is shown in Figure 1.

Concordance between TST and QFN

Agreement between the TST and QFN was poor in the TB patients (kappa 0.09) compared to the community controls (kappa 0.59). Three patients were negative in both QFN and TST (4.4%, 3/68), and 25% of all TB patients (17/68) had discordant TST and QFN results (Table II). In a control group of blood donors with no history of TB and with no household contact on TB treatment, a positive TST was observed in 47% (17/36) and a positive QFN in 51% (21/41) (Table I). The number of indeterminate QFN results was 5.0% (12/240) overall, including the healthy blood donors at baseline, and varied between 2.7% and 10% during follow-up with 2.7% (3/110) at baseline, 10% (5/49) at month 2–3 and 5.1% (2/39) at month 7).

Kinetics of QFN in relation to HIV co-infection

The rate of QFN-positive TB patients was significantly reduced during treatment from baseline to 7 months (from 93.8% to 62.5% in HIV-negative/TB and from 70.3% to 33.3% in HIV-positive/TB patients; $p < 0.05$, Table III). Following 2–3 months of anti-TB treatment, the rate of TB patients positive in the QFN decreased from 93.8% to 74% (17/23) in HIV-negative/TB and from 70.3% to 42% (11/26) in HIV-positive/TB patients ($p < 0.05$), and after 7 months there was a significant reduction to an overall QFN positivity of 51.3% (20/39, $p < 0.05$) compared to 81.2% at inclusion (Table III). After 2–3 months of treatment 5.3% (1/19) of HIV-negative/TB patients and 42.9% (9/21) of HIV-positive/TB patients ($p < 0.01$) who had a positive QFN result at inclusion had converted to a negative result, while 10.0% (4/40) converted from a positive to an indeterminate result. The rate of QFN-positive individuals turning negative after 7 months of treatment was still higher among HIV-positive/TB than HIV-negative/TB patients (70%, 7/10 vs 29%, 6/22, $p < 0.05$). Conversion from negative to positive QFN was observed in 2 of 5 HIV-positive/TB patients after 2–3 months of treatment. After completion of the intensive phase of TB treatment, 36.8% (14/38) of the HIV-positive/TB patients were initiated on ART against HIV, while the remaining patients were followed up at the ART clinic. The rate of patients on ART, positive in the QFN at month 7 was increased compared to co-infected TB patients without ART (4/5 vs 3/9), however the number of patients in each group was very small.

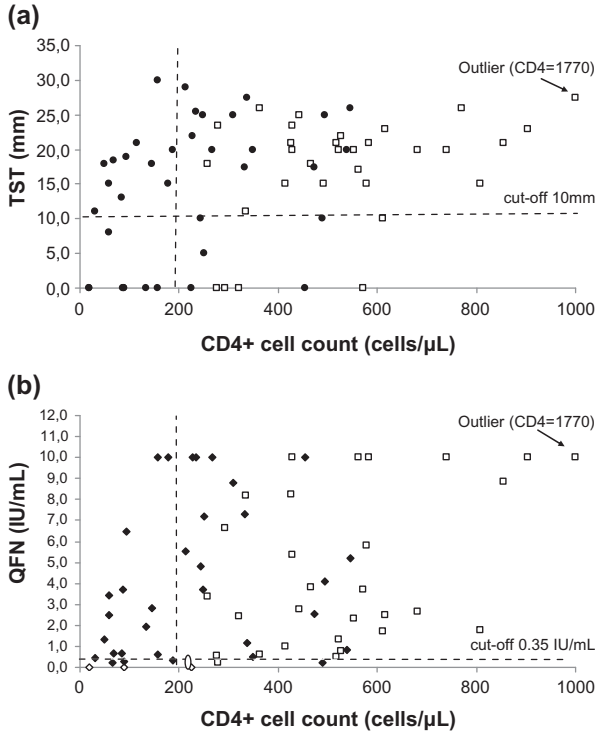

Figure 1. (a) TST (mm) and (b) QuantiFERON-TB Gold In-Tube (IU/ml IFN-γ) at inclusion in relation to CD4+ cell count (cells/μl) for both HIV-positive (filled dots) and HIV-negative (unfilled squares) tuberculosis patients. The cut-off values for a positive test for TST and QFN are indicated with the horizontal lines. Only patients for whom CD4+ cell counts and QFN (n = 65) or TST (n = 64) were available are plotted. The only indeterminate QFN result is plotted as an open circle (224 CD4+ cells/μl).

QFN and TST results at diagnosis in relation to clinical outcome and initial severity of disease

Multiple logistic regression analysis showed a significant correlation between a negative TST initially

Table II. Concordance between TST and QuantiFERON®-TB Gold In-Tube results.

	TST	QFN-pos	QFN-neg	QFN indeterminate
HIV-neg/TB	Positive	27	1	0
(n = 32)	Negative	3	1	0
HIV-pos/TB	Positive	20	7	0
(n = 36)	Negative	6	2	1
CC	Positive	13	3	1
(n = 36)	Negative	4	14	1

TST, tuberculin skin test; QFN, QuantiFERON-TB Gold In-Tube; HIV, human immunodeficiency virus; TB, tuberculosis; CC, community control.
Only cases with complete data available for both TST and QFN were included.

and a more advanced TB measured as the TB score (adjusted odds ratio 30.7; 95% confidence interval 1.52–618.2; p = 0.026), which was not confounded by factors such as HIV, age or sex. Moreover, TST-negative patients had lower cure rates, higher mortality and an increased smear-positive rate at week 8 compared to TST-positive patients, although this did not reach statistical significance (Table III). There was no correlation between a negative QFN test and the severity of disease or the final treatment outcome (Table III).

Discussion

We have investigated the kinetics of the QFN test in a high endemic area for TB and HIV, and the relationship between QFN and TST and the severity of disease, as there is a lack of studies on these aspects. There are few, if any previous reports on the correlation between QFN reactivity, TST and the clinical severity of disease, although it is well known that there are discrepancies between QFN and TST during active TB [12], which we could also confirm. To study the clinical severity of TB, we used a previously validated scoring system for TB—the TB score [13]. We found that a negative TST correlated to a high initial TB score indicating more severe disease, which was not observed in QFN-negative individuals despite similar levels of CD4+ cells. Importantly, this indicates that the severity of the disease may not be an essential factor for a patient with active TB to test negative for QFN. Moreover, this observation further underlines the clinical differences between the tests during active TB and might reflect that TST-responses are less sensitive than QFN in more advanced TB. We found no correlation between patients with a negative QFN and final treatment outcome or sputum smear conversion, although a negative TST showed a tendency to a poor clinical outcome (Table III). In contrast, Katiyar et al. [11] found in a study of 76 patients with active TB that an increased or persistent IFN-γ response at 2 months was associated with a 17.3 (p = 0.007) times higher likelihood of remaining culture-positive at 2 months. However, both studies are limited by the small sample size and a loss to follow-up of QFN. Taken together our data indicate that a negative TST is associated with a poor prognosis and more severe disease, which is not the case with patients exhibiting a negative QFN test.

As one of the few published studies describing the kinetics of QFN results during treatment of active pulmonary TB in a high endemic area, we found a significant decrease following 7 months of anti-TB treatment in HIV-negative/TB and HIV-positive/TB patients. These findings are in agreement with those of Kobashi et al. [9] who found that the rate of QFN-positive decreased from 83% (n = 40) at treatment initiation to 58% at treatment completion (6 months), and with Domínguez et al. [8] who found a sensitivity of 69% (25/36) for QFN in patients with pulmonary TB at treatment initiation compared to patients during treatment, where 49% (21/43) were positive. Transient responses in serial testing with QFN have been reported such as a 33% (5/15) reversion and 6% (3/48) conversion after 3 months [14] and 22% (6/27) discordant result in healthcare workers after 3 days [15]. It has also been described that in patients with QFN levels in close relation to the cut-off (0.2–0.7 IU/ml) the test is vulnerable to bias and reversions, which might not correlate to biological changes [16]. However, it is unlikely that transient responses have affected our results from the kinetic QFN investigation significantly, as we studied active TB which had high QFN responses generally. Furthermore, the QFN results were stable during treatment at month 2–3 and month 7. It is more likely that the observed decrease is explained by the effect of anti-TB treatment by which the immune response to TB antigen is reduced and thereby altering an IFN-γ response below the threshold for a positive QFN reaction. On the other hand a conversion from negative to positive QFN during anti-TB treatment can be a sign of a recovering immune response. In our study the rate of QFN-positive individuals turning negative was higher among HIV-positive/TB patients than HIV-negative/TB patients (month 2–3: 42.9% vs 5.3%, and month 7: 70% vs 29%, $p < 0.05$). Conversion from negative to positive QFN was only observed in 2 of 5 HIV-positive/TB patients after 2–3 months of treatment. One reason could be that other aspects than the CD4+ T-cell count of host immunity are responsible for this effect, such as an increased turnover of T-cells or decreased functionality of this subset. Another factor that could affect the rate of QFN conversion following treatment is ART, but our study sample is too small to come to any conclusions in this regard (Table III).

Table III. TST and QuantiFERON-TB® Gold In-Tube results in relation to clinical outcome, severity and CD4 counts.

	Week 0										Month 7				
	PPD					QFN					QFN				
	Positive	*n*	Negative	*n*	*p*	Positive	*n*	Negative	*n*	*P*[a]	Positive	*n*	Negative	*n*	p[a]
All TB (%)	80.0	56	20.0	14		81.2[b]	56	17.4	12		51.3[b]	20	43.6	17	
TB score week 0	7.1 ± 2.2	49	9.1 ± 1.3	13	0.003	7.6 ± 2.2	50	6.9 ± 2.0	10	NS	7.9 ± 2.2	18	9.1 ± 1.3	12	NS
CD4+ count (cells/µl)	423 ± 289	53	216 ± 159	14	0.01	412 ± 294	54	216 ± 137	11	0.03	542 ± 365	18	293 ± 180	12	0.02
Sputum conversion week 8 (%)	78.4	51	63.6	11	NS	78.9	52	75	8	NS	73.7	19	86.7	15	NS
Cured (%)	80.4	56	57.1	14	0.07	78.6	56	66.7	12	NS	100	20	100	17	0.07
Died (%)	8.9	56	21.4	14	NS	12.5	56	0	12	NS	0	10	5.9	17	NS
HIV-negative/TB (%)	87.9	29	12.1	4		93.8	30	6.2	2		62.5	15	33.3	8	
TB score week 0	7.3 ± 2.3	24	9.5 ± 1.3	4		7.6 ± 2.3	27	9.5	1		8.1±2.8	13	6.6 ± 1.7	5	NS
CD4+ count (cells/µl)	590 ± 289	27	365 ± 139	4		583 ± 287	28	285	2		632±368	15	443 ± 92	7	NS
Sputum conversion week 8 (%)	74.1	27	0	4		78.6	28	0	2		78.6	14	75.0	8	NS
Cured (%)	82.8	29	75.0	4		83.3	30	75.0	2		100	15	100	8	NS
Died (%)	0		0			0		0	10		0		0		
HIV-positive/TB (%)	73.0	27	27.0	10		70.3	26	27.0			33.3	5	60.0	9	
TB-score week 0	6.9 ± 0.82	25	8.9 ± 0.44	9	0.02	7.6 ± 2.2	23	6.7 ± 2.0	9	NS	7.6±2.6	5	7.1 ± 1.5	7	NS
CD4+ count (cells/µl)	249 ± 158	26	156 ± 127	10	NS	229 ± 163	26	200 ± 148	9	NS	271±186	5	176 ± 139	9	NS
Sputum conversion week 8 (%)	83.3	24	42.9	7	0.05	79.2	24	66.7	6	NS	60.0	5	0	7	0.08
Cured (%)	77.8	27	50.0	10	NS	73.1	26	70.0	10	NS	100	5	100	9	NS
Died (%)	18.5	27	30.0	10	NS	26.9	26	40.0	10	NS	0	5	11.1	9	NS
On ART	29.6	27	50.0	10	NS	30.1	26	40.0	10	NS	80.0	5	33.3	9	NS

TST, tuberculin skin test; PPD, purified protein derivative; QFN, QuantiFERON-TB Gold In-Tube; TB, tuberculosis; HIV, human immunodeficiency virus; ART, antiretroviral therapy; NS, not significant.

[a]QFN-positive and QFN-negative patients are compared.

[b]There was 1 QFN indeterminate HIV-positive/TB patient at week 0 and 2 QFN indeterminate patients at month 7 (1 HIV-positive/TB and 1 HIV-negative/TB).

There are also reports that the QFN result can be influenced by the TST in a TST-positive population when a follow-up is performed 2–4 weeks later [17], as well as in serial testing a month after TST among healthy subjects with a TST <15 mm (15% 5/33) [18]; however if tested with QFN the day of TST and the day of TST reading there was no increase in the rate of positive QFN [19]. Nevertheless, the relative contribution of TST testing to boosting in patients diagnosed with clinical TB is likely to be minor and it is unlikely that boosting could be a major part of the explanation of persistent QFN positivity seen in HIV-negative/TB patients in the present study.

The overall sensitivity of the IFN-γ release assay QFN was found to be 81.2% in our study population of 71 sputum smear-positive TB patients, which is similar to the TST using a cut-off for a positive test at ≥10 mm. Our data are comparable to previous published findings from a high TB endemic area in Zambia (QFN positivity 74%, TST positivity 67%) [6] and from Tanzania (overall QFN positivity 74%, HIV-negative/TB 81% and HIV-positive/TB 65%) [7].

Although we did not detect a difference in the overall sensitivity between the TST and QFN, agreement between the 2 methods was poor, which has been described previously [6]. Confirming our data in the community controls, a previous study found fair agreement between the TST (5- and 10-mm cut-offs) and the QFN test (kappa = 0.52–0.6) at least in HIV-positive/TB patients [3]. The TST test is based on the principle that latent or active TB induces a strong cell-mediated immune response that can be measured by the response to an intradermal inoculation of tuberculin purified protein derivative (PPD), a mixture of many M. tuberculosis proteins [1]. Discordant results between IGRAs and the TST cannot be completely explained by the notion that IGRAs are more specific with regard to cross-reaction with non-TB mycobacterial infections or with the BCG vaccine. Although the BCG vaccine coverage of community controls and TB patients in our study was low, there was no correlation between discordant results and the presence of a BCG scar (data not shown).

Estimations of sensitivity and specificity for the QFN are problematic because of the lack of a gold standard for latent infection and because the test cannot separate active from latent disease. This is a particular concern in high endemic areas. Thus, our data from healthy blood donors without any signs of pulmonary TB confirm several reports showing that latent infection measured as QFN reactivity is common (51% (21/41) in this study) in high endemic areas [3]. This underlines that although the QFN has a fairly high sensitivity, the clinical value for diagnosing smear-positive TB in a high endemic area is impaired by the low specificity due to a high background of latent infection. An alternative, as proposed from a study with 216 students in India who underwent testing with TST and QFN, is to modify the optimal thresholds to distinguish infections from non-specific variations [20]. In low TB endemic areas like the Nordic countries, the background prevalence has been described in the range of 2% in HIV-negative and 4% in HIV-positive individuals [4], thus making QFN a more useful diagnostic tool for latent TB in these countries.

HIV co-infection has been described to have an influence on the QFN test, since IFN-γ production might be limited by the reduced number and function of CD4+ T-cells [1]. Moreover, we observed reduced levels of CD4+ T-cells on diagnosis also in HIV-negative/TB patients, which has been described previously. Low CD4+ cell counts have been found to be associated with an increase in both indeterminate and false-negative QFN results in some studies [6,21], but as has been noted in other studies [7], low CD4+ T-cell counts do not seem to account for all indeterminate and negative results in HIV-positive/TB patients. In agreement with this observation we could not detect a significant correlation in the HIV-positive/TB group between negative QFN results and low CD4+ cell counts, including the rate in patients with CD4+ cell counts lower than 200 cells/μl. We found that even very low CD4+ cell counts may result in an IFN-γ response high enough to be detected in the QFN test, suggesting that the quality and function of the present CD4+ T-cells are of importance. Possibly, CD8+ T-cells could contribute to the increased IFN-γ production in patients with low levels of CD4+ T-cells.

We conclude that the QuantiFERON®-TB Gold In-Tube reactivity is significantly reduced at the end of treatment against active TB to the background level of healthy blood donors. In smear-positive TB patients, agreement between the TST and QFN is poor, including correlation to the severity of disease, which is most likely linked to the ability of the tests to measure different aspects of immunity.

Acknowledgements

The authors wish to thank Nurse Meseret Senbeto, Mr Tezera Jemere, Ms Saba Ekubay, Mr Belay Anagaw for invaluable support in monitoring patients and handling patient samples. We also wish to thank the study participants without whom the study would not have been possible. Financial support was received from the Swedish Heart and Lung Foundation, SAREC/SIDA and the Swedish Research Council.

Declaration of interest: No conflict of interest to declare.

References

[1] Lalvani A. Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest 2007;131:1898–906.

[2] Pai M, Zwerling A, Menzies D. Systematic review: T-cell based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med 2008;149:177–84.

[3] Dheda K, Smit RZ, Badri M, Pai M. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. Curr Opin Pulm Med 2009;15:188–200.

[4] Soborg B, Andersen AB, Larsen HK, Weldingh K, Andersen P, Kofoed K, et al. Detecting a low prevalence of latent tuberculosis among health care workers in Denmark detected by M. tuberculosis specific IFN-gamma whole-blood test. Scand J Infect Dis 2007;39:554–9.

[5] Mazurek GH, Weis SE, Moonan PK, Daley CL, Bernardo J, Lardizabal AA, et al. Prospective comparison of the tuberculin skin test and 2 whole-blood interferon-gamma release assays in persons with suspected tuberculosis. Clin Infect Dis 2008;46:474–5; author reply 475.

[6] Raby E, Moyo M, Devendra A, Banda J, De Haas P, Ayles H, et al. The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay in Zambian adults with active tuberculosis. PLoS One 2008;3:e2489.

[7] Aabye MG, Ravn P, PrayGod G, Jeremiah K, Mugomela A, Jepsen M, et al. The impact of HIV infection and CD4 cell count on the performance of an interferon gamma release assay in patients with pulmonary tuberculosis. PLoS One 2009;4:e4220.

[8] Domínguez J, De Souza-Galvão M, Ruiz-Manzano J, Latorre I, Prat C, Lacoma A, et al. T-cell responses to the Mycobacterium tuberculosis-specific antigens in active tuberculosis patients at the beginning, during, and after anti-tuberculosis treatment. Diagn Microbiol Infect Dis 2009;63:43–51.

[9] Kobashi Y, Mouri K, Yagi S, Obase Y, Miyashita N, Oka M. et al. Transitional changes in T-cell responses to Mycobacterium tuberculosis-specific antigens during treatment. J Infect 2009;58:197–204.

[10] Kobashi Y, Sugiu T, Ohue Y, Mouri K, Obase Y, Miyashita N, et al. Long-term follow-up of the QuantiFERON TB-2G test for active tuberculosis disease. Intern Med 2008;47:1957–61.

[11] Katiyar SK, Sampath A, Bihari S, Mamtani M, Kulkarni H. Use of the QuantiFERON TB Gold In-Tube test to monitor treatment efficacy in active pulmonary tuberculosis. Int J Tuberc Lung Dis 2008;12:1146–52.

[12] Adetifa IM, Lugos MD, Hammond A, Jeffries D, Donkor S, Adegbola RA, et al. Comparison of two interferon gamma release assays in the diagnosis of Mycobacterium tuberculosis infection and disease in The Gambia. BMC Infect Dis 2007;7:122.

[13] Wejse C, Gustafson P, Nielsen J, Gomes VF, Aaby P, Andersen PL, et al. TBscore: signs and symptoms from tuberculosis patientsin a low-resource setting have predictive value and may be used to assess clinical course. Scand J Infect Dis 2007;40:2111–20.

[14] Perry S, Sanchez L, Yang S, Agarwal Z, Hurst P, Parsonnet J. Reproducibility of QuantiFERON-TB gold in-tube assay. Clin Vaccine Immunol 2008;15:425–32.

[15] Detjen AK, Loebenberg L, Grewal HM, Stanley K, Gutschmidt A, Kruger C, et al. Short-term reproducibility of a commercial interferon gamma release assay. Clin Vaccine Immunol 2009;16:1170–5.

[16] Choi JC, Shin JW, Kim JY, Park IW, Choi BW, Lee MK. The effect of previous tuberculin skin test on the follow-up examination of whole-blood interferon-gamma assay in the screening for latent tuberculosis infection. Chest 2008;133: 1415–20.

[17] Igari H, Watanabe A, Sato T. Booster phenomenon of QuantiFERON-TB Gold after prior intradermal PPD injection. Int J Tuberc Lung Dis 2007;11:788–91.

[18] Leyten EM, Prins C, Bossink AW, Thijsen S, Ottenhoff TH, van Dissel JT, et al. Effect of tuberculin skin testing on a Mycobacterium tuberculosis-specific interferon-gamma assay. Eur Respir J 2007;29:1212–6.

[19] Pai M, Joshi R, Dogra S, Mendiratta DK, Narang P, Kalantri S, et al. Serial testing of health care workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med 2006; 174:349–55.

[20] Rangaka MX, Wilkinson KA, Seldon R, Van Cutsem G, Meintjes GA, Morroni C, et al. Effect of HIV-1 infection on T-cell-based and skin test detection of tuberculosis infection. Am J Respir Crit Care Med 2006;175:514–20.

[21] Van Zyl-Smit RN, Pai M, Peprah K, Meldau R, Kieck J, Juritz J, et al. Within-subject variability and boosting of T-cell interferon-gamma responses after tuberculin skin testing. Am J Respir Crit Care Med 2009;180:49–58.